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L3: Entry 1 of 1

File: PGPB

Jun 26, 2003

DOCUMENT-IDENTIFIER: US 20030118566 A1

TITLE: Compositions and methods for isolation, propagation, and differentiation of human stem cells and uses thereof

Detail Description Paragraph:

[0182] Animals were sacrificed 60 days after transplantation, brains sectioned and immunostained. Sections were incubated after blocking with 3% BSA and 0.02% Tween 20 for 16 hours at 4.degree. C. All primary antibodies were diluted in PBS containing 3% BSA and 0.02% Tween 20. Antibodies that were used in this study were anti-human nucleus antibodies (1:50, Chemicon) to detect human cells, antitype III .beta.-tubulin antibodies (1:100, Chemicon) to detect neurons, anti tyrosine hydroxylase (1:100 Sigma) and dopa decarboxylase (1:200, Chemicon) antibodies to detect dopaminergic cells, anti gamma amino acid decarboxylase (GAD) antibodies to identify GABAergic neurons (1:1000, Chemicon), anti L-glutamate antibodies to detect glutamatergic neurons (1:50, Signature Immunologics), anti-glycine antibodies to detect glycinergic neurons (1:100, Signature Immunologics), anticholine acetyl transferase (CHAT) to detect cholinergic neurons (1:100 Chemicon) and anti GFAP antibodies (1:500, DAKO) to detect astrocytes. For double staining sections were incubated simultaneously with two primary and secondary antibodies. The second antibodies were goat anti-mouse FITC (1:200, Sigma) and goat anti-rabbit rhodamine (1:200, Boehringer).

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L5: Entry 24 of 24

File: USPT

Jun 6, 1995

DOCUMENT-IDENTIFIER: US 5422110 A

TITLE: Enhanced immunogenicity using leukotoxin chimeras

Detailed Description Text (7):

The term "leukotoxin polypeptide" intends a polypeptide derived from a protein belonging to the family of molecules characterized by the carboxy-terminus consensus amino acid sequence Gly-Gly-X-Gly-X-Asp (SEQ ID NO:11) (Highlander et al., DNA (1989) 8:15-28), where X is Lys, Asp, Val or Asn. Such proteins include, among others, leukotoxins derived from *P. haemolytica* and *Actinobacillus pleuropneumoniae*, as well as *E. coli* alpha hemolysin (Strathdee, C. A., and Lo, R. Y. C. Infect. Immun. (1987) 55:3233-3236; Lo, R. Y. C., Can. J. Vet. Res. (1990) 54:S33-S35; Welch, R. A., Mol. Microbiol. (1991) 5:521-528). This family of toxins is known as the "RTX" family of toxins (Lo, R. Y. C., Can. J. Vet. Res. (1990) 54:S33-S35). In addition, the term "leukotoxin polypeptide" refers to a leukotoxin polypeptide which is chemically synthesized, isolated from an organism expressing the same, or recombinantly produced. Furthermore, the term intends an immunogenic protein having an amino acid sequence substantially homologous to a contiguous amino acid sequence found in the particular native leukotoxin molecule. Thus, the term includes both full-length and partial sequences, as well as analogs. Although native full-length leukotoxins display leukotoxic activity, the term "leukotoxin" also intends molecules which remain immunogenic yet lack the cytotoxic character of native leukotoxins. The nucleotide sequences and corresponding amino acid sequences for several leukotoxins are known. See, e.g., U.S. Pat. Nos. 4,957,739 and 5,055,400; Lo et al., Infect. Immun. (1985) 50:667-67; Lo et al., Infect. Immun. (1987) 55:1987-1996; Strathdee, C. A., and Lo, R. Y. C., Infect. Immun. (1987) 55:3233-3236; Highlander et al., DNA (1989) 8:15-28; Welch, R. A., Mol. Microbiol. (1991) 5:521-528.

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L1: Entry 2 of 2

File: USPT

Jan 14, 1997

DOCUMENT-IDENTIFIER: US 5594107 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Chimeric protein comprising an RTX-family cytotoxin and interferon-2 or interferon

Detailed Description Text (7):

The term "RTX cytotoxin" intends a cytotoxin belonging to the family of cytolytic toxins known as the RTX proteins. The toxins are characterized by a series of repeated amino acid domains near the carboxy terminus. The consensus amino acid sequence is Gly-Gly-X-Gly-(Asn/Asp)-Asp (SEQ ID NO: 5), where X is Lys, Asp, Val or Asn. Such proteins include, among others, leukotoxins derived from *Pasteurella* and *Actinobacillus*, such as those found in *P. haemolytica*, *Actinobacillus pleuropneumoniae*, *A. actinomycetemcomitans*, *A. suis*, as well as the 0 cytotoxins found in *Proteus vulgaris*, *Morganella morganii*, *Moraxella bovis*, *Neisseria meningitidis*, *H. influenzae* type B, *E. coli* alpha hemolysin and *Bordetella pertussis* adenylate cyclase hemolysin. (For further descriptions of these toxins, see, e.g., Strathdee, C. A., and Lo, R. Y. C. (1987) *Infect. Immun.* 55: 3233-3236; Lo, R. Y. C. (1990) *Can. J. Vet. Res.* 54: S33-S35; Welch, R. A. (1991) *Mol. Microbiol.* 5: 521-528; Lo et al. (1987) *Infect. Immun.* 55: 1987-1996; Glaser et al. (1988) *Molec. Microbiol.* 2: 19-30; Lally et al. (1989) *J. Biol. Chem.* 254: 15451-15456; Kolodrubetz et al. (1989) *Infect. Immun.* 57: 1465-1469; Chang et al. (1989) *DNA* 8: 635-647; Frey, J. and Nicolet, J. (1988) *Infect. Immun.* 56: 2570-2575; Devenish et al. (1989) *Infect. Immun.* 57: 3210-3213; Koronakis et al. (1987) *J. Bacteriol.* 169: 1509-1515 and Highlander et al. (1989) *DNA* 8: 15-28). The desired cytotoxin may be chemically synthesized, isolated from an organism expressing the same, or recombinantly produced.

Detailed Description Text (10):

The term "epitope" refers to the site on an antigen or hapten to which a specific antibody molecule binds. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." One such epitope is the consensus sequence found among the RTX family of toxins described above. This sequence is Gly-Gly-X-Gly-(Asn/Asp)-Asp (SEQ ID NO: 5), where X is preferably Lys, Asp, Val or Asn. Other substitutions for X in the consensus sequence are also contemplated including substitutions with an aliphatic amino acid, such as Gly, Ala, Val, Leu, Ile, a charged amino acid such as Asp, Glu, Arg, His or Lys, or a corresponding neutral amino acid such as Asn or Gln.

Detailed Description Text (126):

As explained above, the *P. haemolytica* leukotoxin protein is a member of the RTX family of toxins and contains a series of repeated amino acid domains near the carboxy terminus. These domains are likely to be epitopes useful in the subject chimeric proteins. The consensus amino acid sequence is Gly-Gly-X-Gly-(Asn or Asp)-Asp (SEQ ID NO: 5), where X is Lys, Asp, Val or Asn. (Highlander et al. (1989) *DNA* 8: 15-28; Welch, R. A. (1991) *Molec. Microbiol.* 5: 521-528). However, other substitutions likely to render immunologically active peptides include substitutions with an aliphatic amino acid, such as Gly, Ala, Val, Leu, Ile, a charged amino acid such as Asp, Glu, Arg, His or Lys, or a corresponding neutral amino acid such as Asn or Gln.

## CLAIMS:

1. An immunogenic chimeric protein comprising a cytokine selected from the group consisting of interleukin-2 (IL2), and gamma-interferon (gamma-IFN), linked to at least one epitope of an RTX cytotoxin which comprises the amino acid sequence Gly-Gly-X-Gly-(Asn or Asp)-Asp (SEQ ID NO: 5), wherein X is selected from the group consisting of an aliphatic amino acid, and a charged amino acid or

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L1: Entry 1 of 2

File: USPT

Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096320 A

TITLE: Vaccines with chimeric protein comprising gamma-interferon and leukotoxin derived from pasteurilla haemolytica

Detailed Description Text (8):

The term "RTX cytotoxin" intends a cytotoxin belonging to the family of cytolytic toxins known as the RTX proteins. The toxins are characterized by a series of repeated amino acid domains near the carboxy terminus. The consensus amino acid sequence is Gly-Gly-X-Gly-(Asn/Asp)-Asp (SEQ ID NO:5), where X is Lys, Asp, Val or Asn. Such proteins include, among others, leukotoxins derived from *Pasteurella* and *Actinobacillus*, such as those found in *P. haemolytica*, *Actinobacillus pleuropneumoniae*, *A. actinomycetemcomitans*, *A. suis*, as well as the cytotoxins found in *Proteus vulgaris*, *Morganella morganii*, *Moraxella bovis*, *Neisseria meningitidis*, *H. influenzae* type B, *E. coli* alpha hemolysin and *Bordetella pertussis* adenylate cyclase hemolysin. (For further descriptions of these toxins, see, e.g., Strathdee, C. A., and Lo, R. Y. C. (1987) *Infect. Immun.* 55:3233-3236; Lo, R. Y. C. (1990) *Can. J. Vet. Res.* 54:S33-S35; Welch, R. A. (1991) *Mol. Microbiol.* 5:521-528; Lo et al. (1987) *Infect. Immun.* 55:1987-1996; Glaser et al. (1988) *Molec. Microbiol.* 2:19-30; Lally et al. (1989) *J. Biol. Chem.* 254:15451-15456; Kolodrubetz et al. (1989) *Infect. Immun.* 57:1465-1469; Chang et al. (1989) *DNA* 8:635-647; Frey, J. and Nicolet, J. (1988) *Infect. Immun.* 56:2570-2575; Devenish et al. (1989) *Infect. Immun.* 57:3210-3213; Koronakis et al. (1987) *J. Bacteriol.* 169:1509-1515 and Highlander et al. (1989) *DNA* 8:15-28). The desired cytotoxin may be chemically synthesized, isolated from an organism expressing the same, or recombinantly produced.

Detailed Description Text (11):

The term "epitope" refers to the site on an antigen or hapten to which a specific antibody molecule binds. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site." One such epitope is the consensus sequence found among the RTX family of toxins described above. This sequence is Gly-Gly-X-Gly-(Asn/Asp)-Asp (SEQ ID NO:5), where X is preferably Lys, Asp, Val or Asn. Other substitutions for X in the consensus sequence are also contemplated including substitutions with an aliphatic amino acid, such as Gly, Ala, Val, Leu, Ile, a charged amino acid such as Asp, Glu, Arg, His or Lys, or a corresponding neutral amino acid such as Asn or Gln.

Detailed Description Text (131):

As explained above, the *P. haemolytica* leukotoxin protein is a member of the RTX family of toxins and contains a series of repeated amino acid domains near the carboxy terminus. These domains are likely to be epitopes useful in the subject chimeric proteins. The consensus amino acid sequence is Gly-Gly-X-Gly-(Asn or Asp)-Asp, (SEQ ID NO:5) where X is Lys, Asp, Val or Asn. (Highlander et al. (1989) *DNA* 8:15-28; Welch, R. A. (1991) *Molec. Microbiol.* 5:521-528). However, other substitutions likely to render immunologically active peptides include substitutions with an aliphatic amino acid, such as Gly, Ala, Val, Leu, Ile, a charged amino acid such as Asp, Glu, Arg, His or Lys, or a corresponding neutral amino acid such as Asn or Gln.